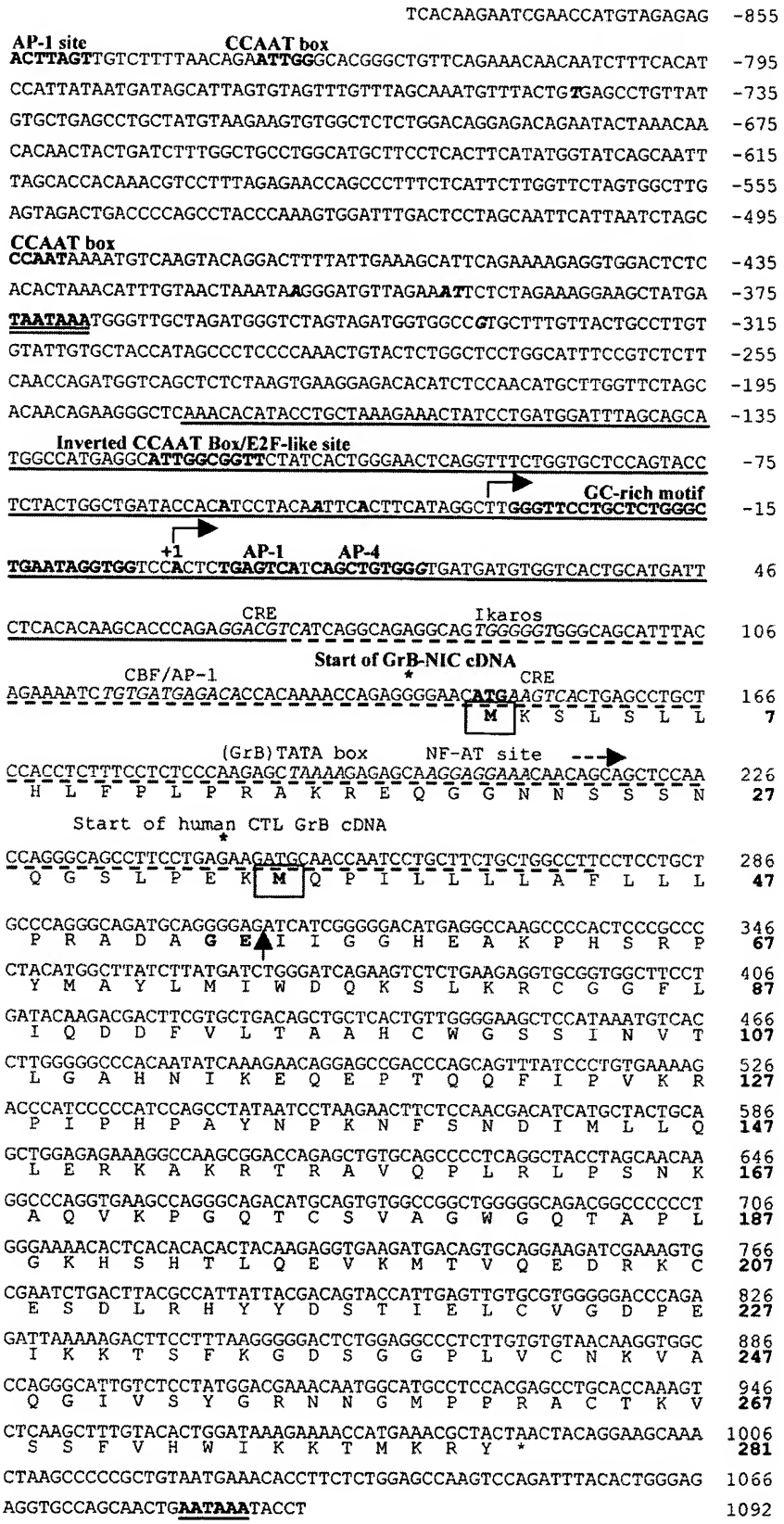
TCACAAGAATCGAACCATGTAGAGAG -855
AP-1 site
CCAAT box
ACTTAGTTGTCTTTTAACAGAATTGGGCACGGGCTGTTCAGAAACAACAATCTTTCACAT -795
CCATTATAATGATAGCATTAGTGTAGTTTGTTTAGCAAATGTTTACTGTGAGCCTGTTAT -735
GTGCTGAGCCTGCTATGTAAGAAGTGTGGCTCTCTGGACAGGAGACAGAATACTAAACAA -675
CACAACTACTGATCTTTGGCTGCCTGGCATGCTTCCTCACTTCATATGGTATCAGCAATT -615
TAGCACCACAAACGTCCTTTAGAGAACCAGCCCTTTCTCATTCTTGGTTCTAGTGGCTTG -555
AGTAGACTGACCCCAGCCTACCCAAAGTGGATTTGACTCCTAGCAATTCATTAATCTAGC -495
CCAAT box
CCAATAAAATGTCAAGTACAGGACTTTTATTGAAAGCATTCAGAAAAGAGGTGGACTCTC -435
ACACTAAACATTTGTAACTAAATAAGGGATGTTAGAAATTCTCTAGAAAGGAAGCTATGA -375
TAATAAATGGGTTGCTAGATGGGTCTAGTAGATGGTGGCCGTGCTTTGTTACTGCCTTGT -315
GTATTGTGCTACCATAGCCCTCCCCAAACTGTACTCTGGCTCCTGGCATTTCCGTCTCTT -255
CAACCAGATGGTCAGCTCTCTAAGTGAAGGAGACACATCTCCAACATGCTTGGTTCTAGC -195
ACAACAGAAGGGCTCAAACACATACCTGCTAAAGAAACTATCCTGATGGATTTAGCAGCA -135
Inverted CCAAT Box/E2F-like site
TGGCCATGAGGCATTGGCGGTTCTATCACTGGGAACTCAGGTTTCTGGTGCTCCAGTACC -75
GC-rich motif
TCTACTGGCTGATACCACATCCTACAATTCACTTCATAGGCTTGGGTTCCTGCTCTGGGC -15
+1
AP-1
AP-4
TGAATAGGTGGTCCACTCTGAGTCATCAGCTGTGGGTGATGATGTGGTCACTGCATGATT 46
CRE
Ikaros
CTCACACAAGCACCCAGAGGACGTCATCAGGCAGAGGCAGTGGGGGTGGGCAGCATTTAC 106
Start of GrB-NIC cDNA
CBF/AP-1
CRE
AGAAAATCTGTGATGAGACACCACAAAACCAGAGGGGAACATGAAGTCACTGAGCCTGCT 166
M K S L S L L 7
(GrB) TATA box
NF-AT site
CCACCTCTTTCCTCTCCCAAGAGCTAAAAGAGAGCAAGGAGGAAACAACAGCAGCTCCAA 226
H L F P L P R A K R E Q G G N N S S S N 27
Start of human CTL GrB cDNA
CCAGGGCAGCCTTCCTGAGAAGATGCAACCAATCCTGCTTCTGCTGGCCTTCCTCCTGCT 286
Q G S L P E K M Q P I L L L L A F L L L 47
GCCCAGGGCAGATGCAGGGGAGATCATCGGGGGACATGAGGCCAAGCCCCACTCCCGCCC 346
P R A D A G E I I G G H E A K P H S R P 67
CTACATGGCTTATCTTATGATCTGGGATCAGAAGTCTCTGAAGAGGTGCGGTGGCTTCCT 406
Y M A Y L M I W D Q K S L K R C G G F L 87
GATACAAGACGACTTCGTGCTGACAGCTGCTCACTGTTGGGGAAGCTCCATAAATGTCAC 466
I Q D D F V L T A A H C W G S S I N V T 107
CTTGGGGGCCCACAATATCAAAGAACAGGAGCCGACCCAGCAGTTTATCCCTGTGAAAAG 526
L G A H N I K E Q E P T Q Q F I P V K R 127
ACCCATCCCCCATCCAGCCTATAATCCTAAGAACTTCTCCAACGACATCATGCTACTGCA 586
P I P H P A Y N P K N F S N D I M L L Q 147
GCTGGAGAGAAAGGCCAAGCGGACCAGAGCTGTGCAGCCCCTCAGGCTACCTAGCAACAA 646
L E R K A K R T R A V Q P L R L P S N K 167
GGCCCAGGTGAAGCCAGGGCAGACATGCAGTGTGGCCGGCTGGGGGCAGACGGCCCCCCT 706
A Q V K P G Q T C S V A G W G Q T A P L 187
GGGAAAACACTCACACACACTACAAGAGGTGAAGATGACAGTGCAGGAAGATCGAAAGTG 766
G K H S H T L Q E V K M T V Q E D R K C 207
CGAATCTGACTTACGCCATTATTACGACAGTACCATTGAGTTGTGCGTGGGGGACCCAGA 826
E S D L R H Y Y D S T I E L C V G D P E 227
GATTAAAAAAGACTTCCTTTAAGGGGGACTCTGGAGGCCCTCTTGTGTGTAACAAGGTGGC 886
I K K T S F K G D S G G P L V C N K V A 247
CCAGGGCATTGTCTCCTATGGACGAAACAATGGCATGCCTCCACGAGCCTGCACCAAAGT 946
Q G I V S Y G R N N G M P P R A C T K V 267
CTCAAGCTTTGTACACTGGATAAAGAAAACCATGAAACGCTACTAACTACAGGAAGCAAA 1006
S S F V H W I K K T M K R Y * 281
CTAAGCCCCCGCTGTAATGAAACACCTTCTCTGGAGCCAAGTCCAGATTTACACTGGGAG 1066
AGGTGCCAGCAACTGAATAAATACCT 1092

Figure 1

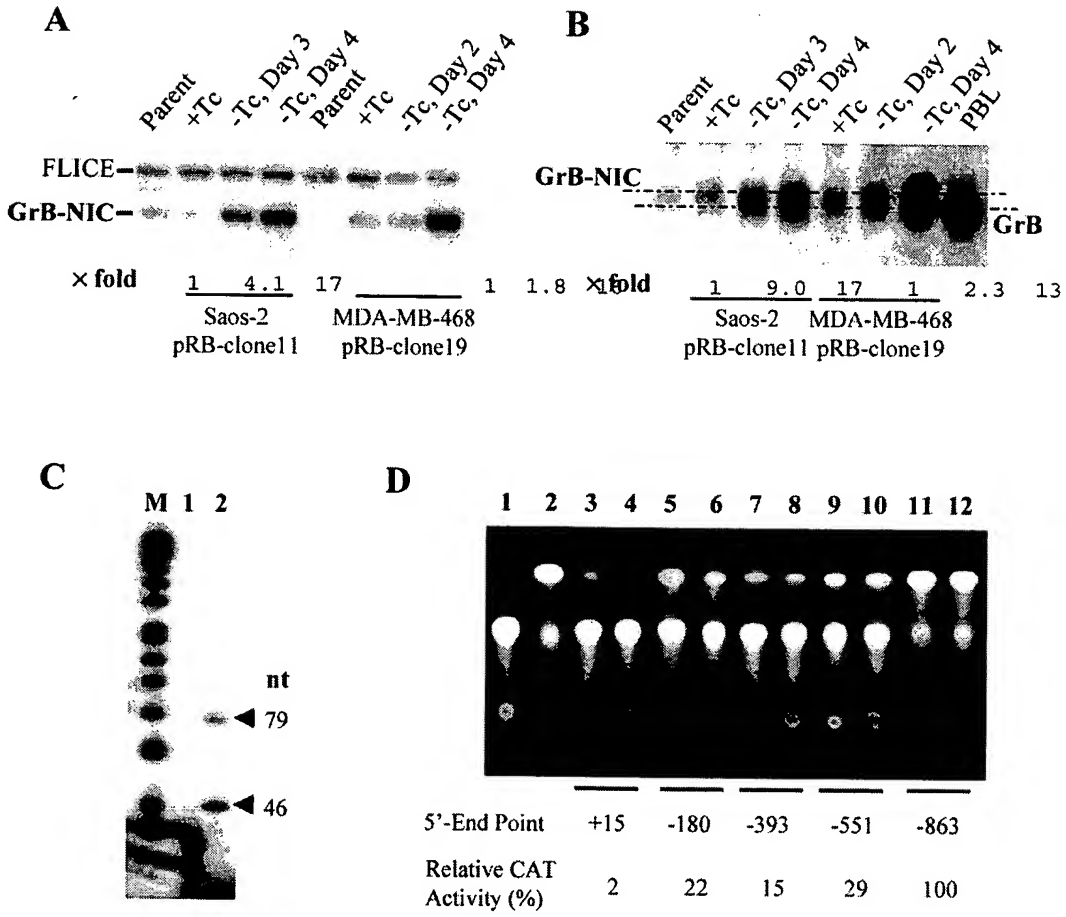
A
Parent
+Tc
-Tc, Day 3
-Tc, Day 4
Parent
+Tc
-Tc, Day 2
-Tc, Day 4
FLICE
GrB-NIC
× fold
1 4.1 17 1 1.8
Saos-2 pRB-clone11
MDA-MB-468 pRB-clone19
B
Parent
+Tc
-Tc, Day 3
-Tc, Day 4
+Tc
-Tc, Day 2
-Tc, Day 4
PBL
GrB-NIC
GrB
× fold
1 9.0 17 1 2.3 13
Saos-2 pRB-clone11
MDA-MB-468 pRB-clone19
C
M 1 2
nt
79
46
D
1 2 3 4 5 6 7 8 9 10 11 12
5'-End Point +15 -180 -393 -551 -863
Relative CAT Activity (%) 2 22 15 29 100

Figure 2

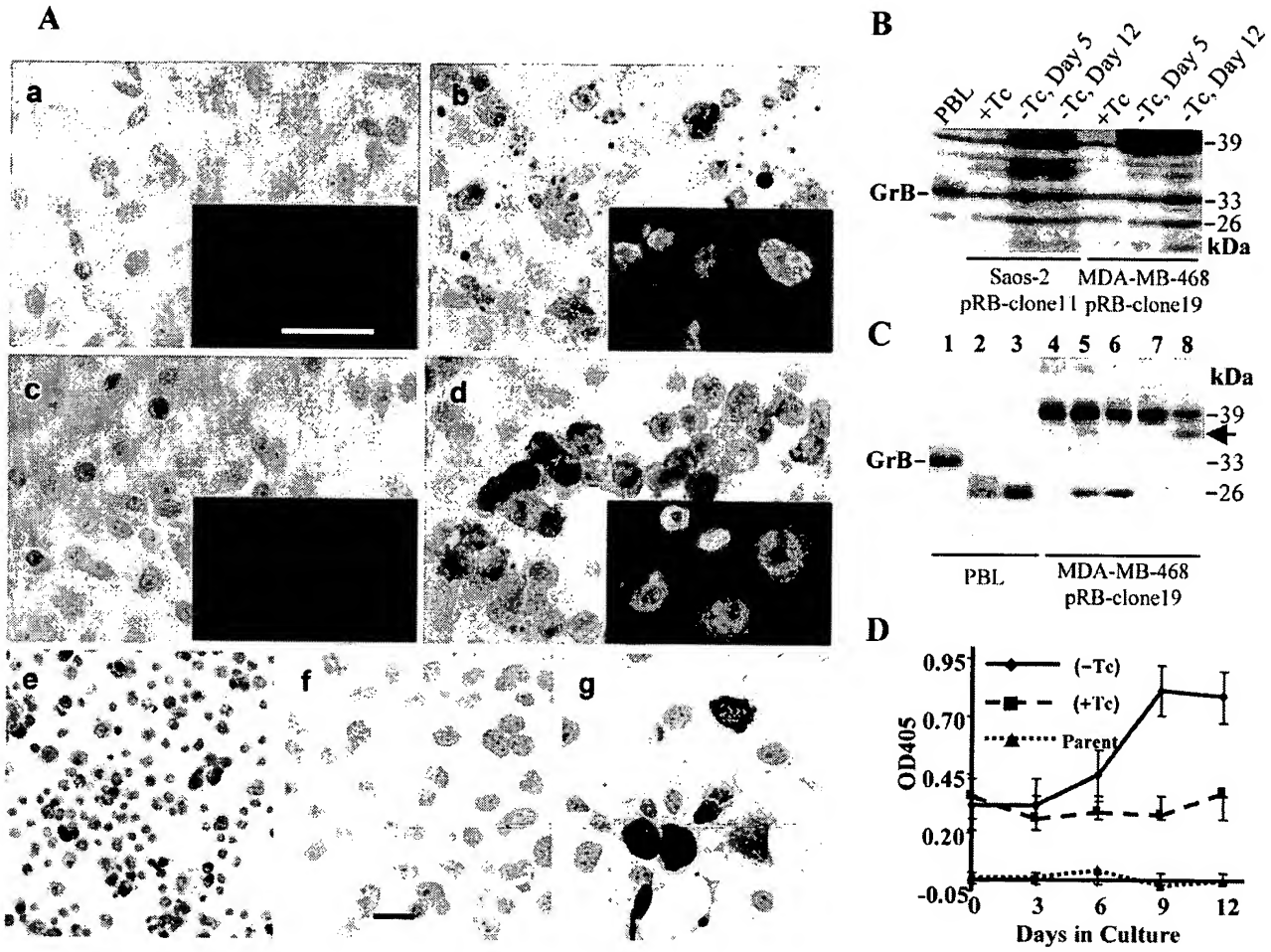
A
a
b
c
d
e
f
g
B
PBL
+Tc
-Tc, Day 5
-Tc, Day 12
+Tc
-Tc, Day 5
-Tc, Day 12
GrB-
39
33
26
kDa
Saos-2
pRB-clone11
MDA-MB-468
pRB-clone19
C
1 2 3 4 5 6 7 8
kDa
GrB-
39
33
26
PBL
MDA-MB-468
pRB-clone19
D
0.95
0.70
0.45
0.20
-0.05
OD405
(-Tc)
(+Tc)
Parent
0
3
6
9
12
Days in Culture

Figure 3

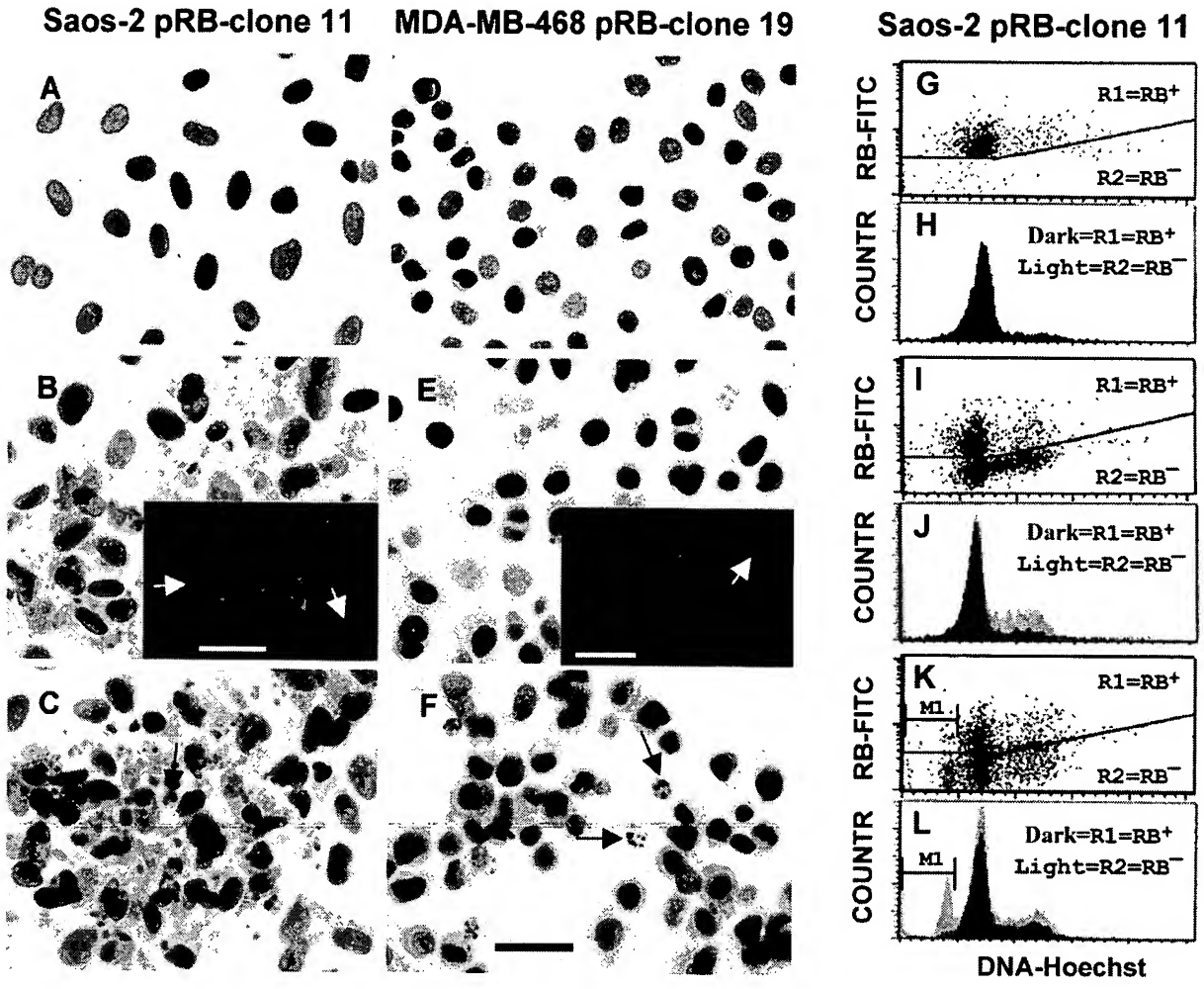
Saos-2 pRB-clone 11
MDA-MB-468 pRB-clone 19
Saos-2 pRB-clone 11
A
B
C
D
E
F
G
H
I
J
K
L
RB-FITC
COUNTR
R1=RB+
R2=RB−
Dark=R1=RB+
Light=R2=RB−
M1
DNA-Hoechst

Figure 4

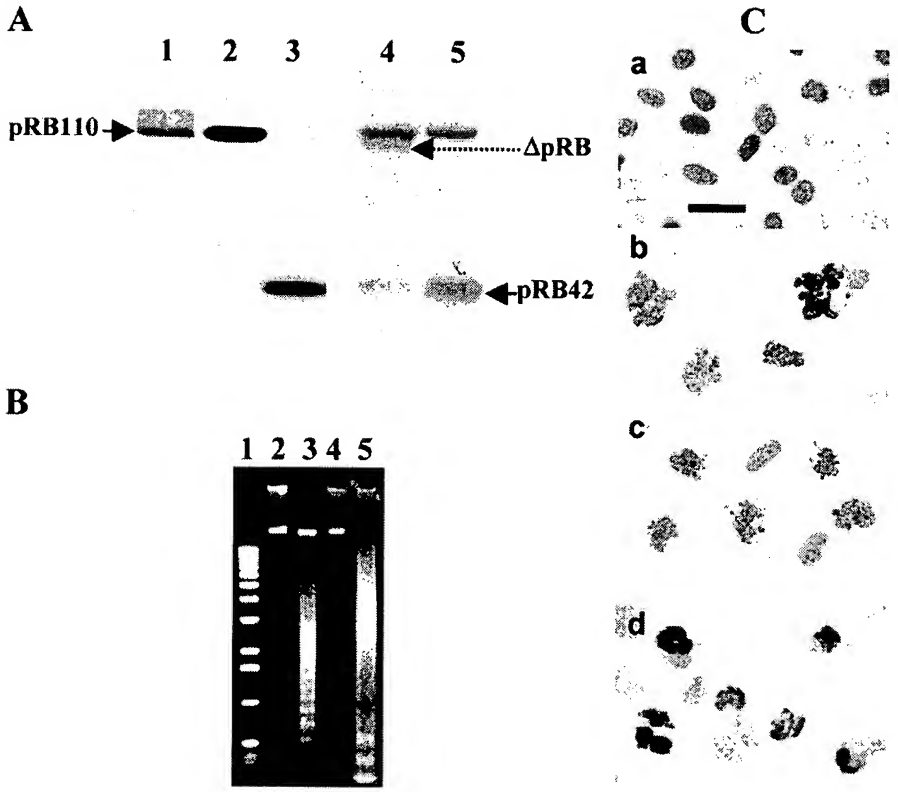

Figure 5

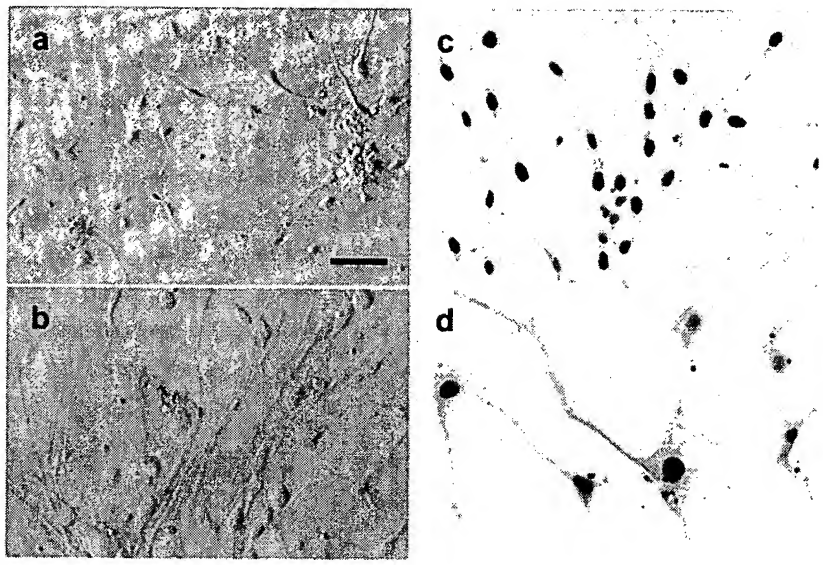

Figure 6

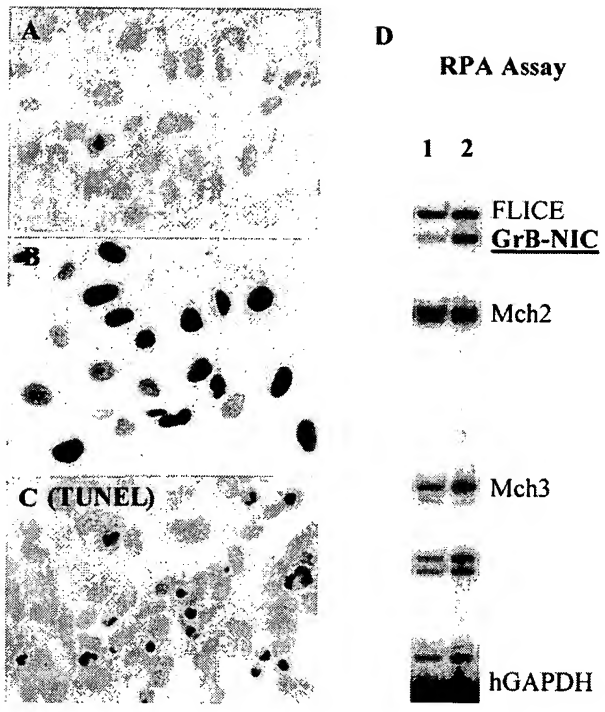

Figure 7

MDA-MB-468 pRB-clone 19

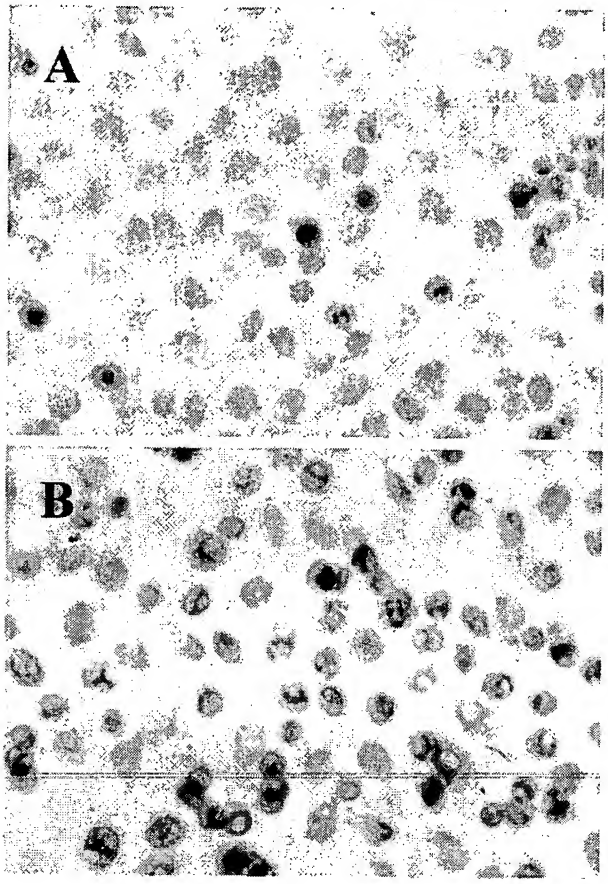

Figure 8

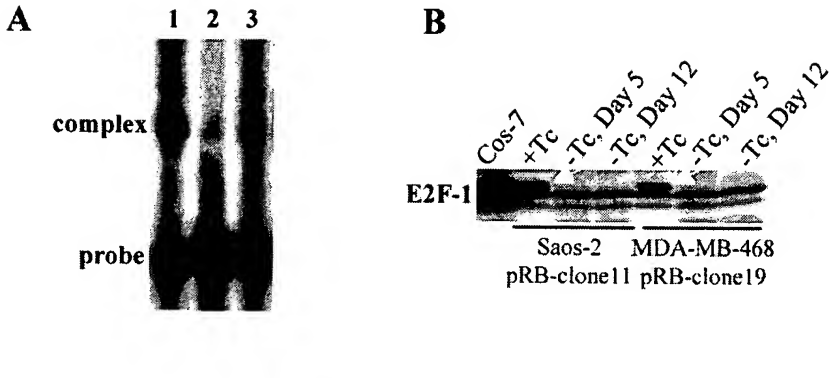
A
1 2 3
complex
probe
B
Cos-7
+Tc
-Tc, Day 5
-Tc, Day 12
+Tc
-Tc, Day 5
-Tc, Day 12
E2F-1
Saos-2
MDA-MB-468
pRB-clone11
pRB-clone19

Figure 9

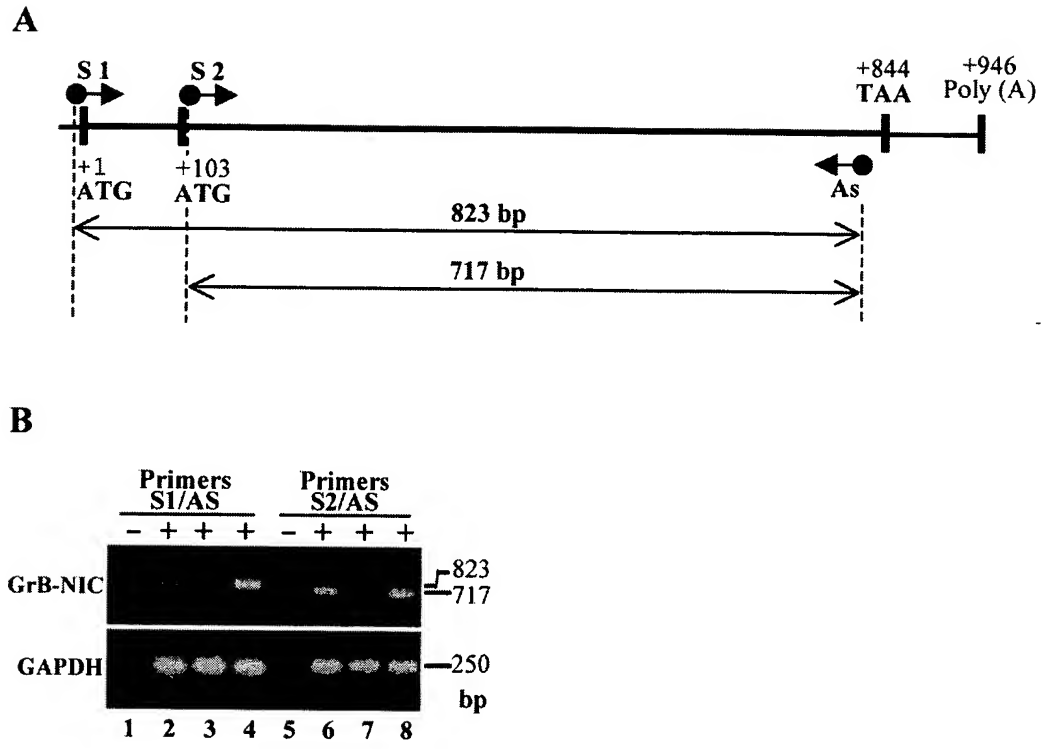
A
S 1
S 2
+844
TAA
+946
Poly (A)
+1
ATG
+103
ATG
As
823 bp
717 bp
B
Primers
S1/AS
Primers
S2/AS
- + + + - + + +
GrB-NIC
823
717
GAPDH
250
bp
1 2 3 4 5 6 7 8

Figure 10

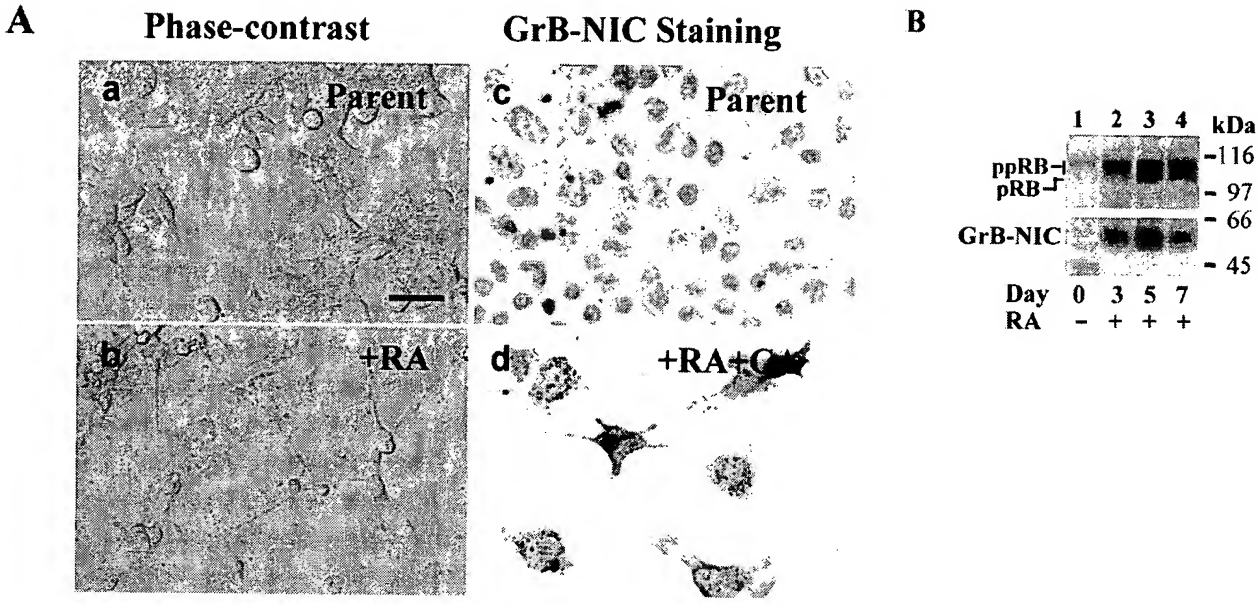
A
Phase-contrast
GrB-NIC Staining
B
a
Parent
c
Parent
b
+RA
d
+RA+C
1 2 3 4
kDa
ppRB
pRB
116
97
66
GrB-NIC
45
Day 0 3 5 7
RA - + + +

Figure 11

Breeding Scheme:

The RB and GrB-NIC double-mutant mouse embryos extended survival to approximately embryonic day 19.5

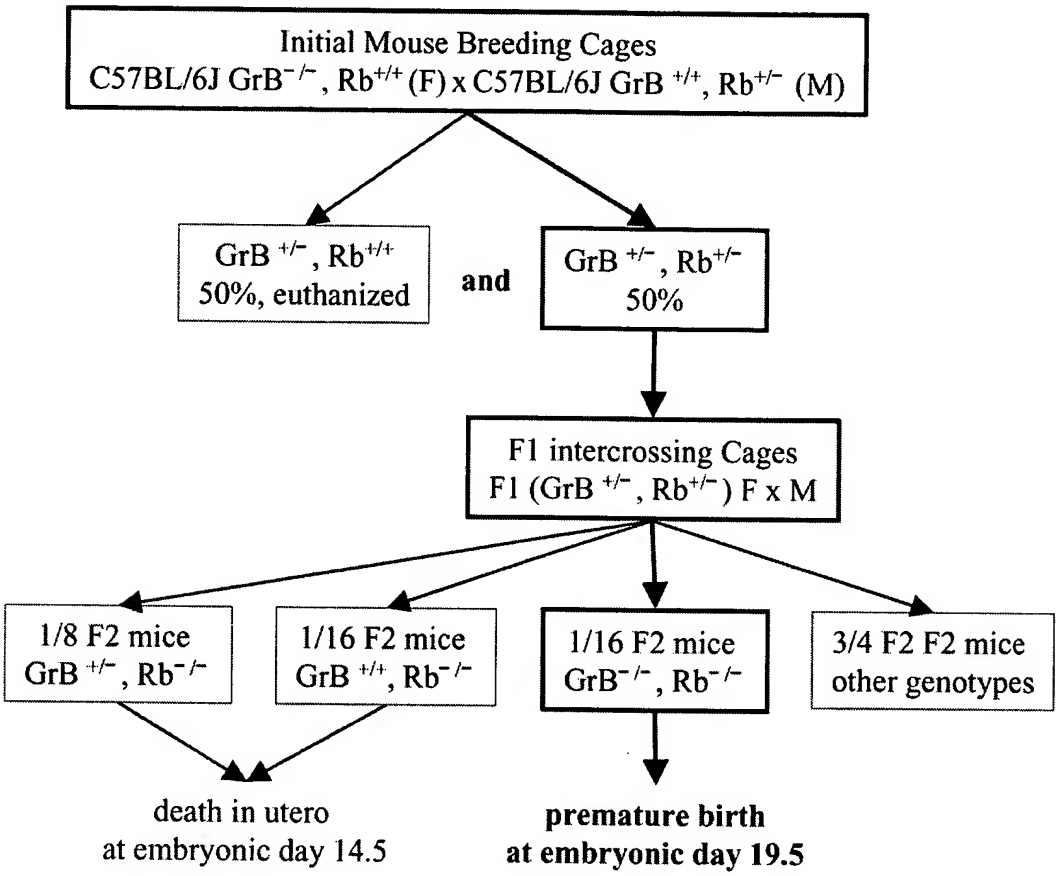

Figure 12

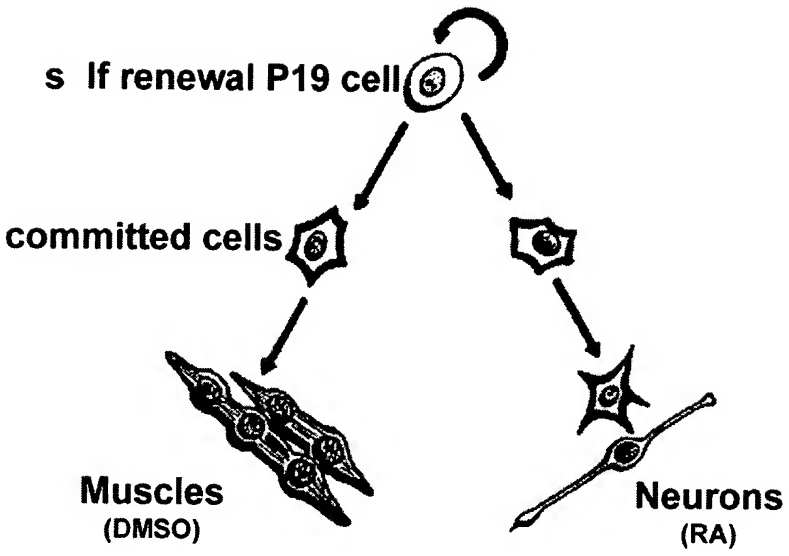
s lf renewal P19 cell
committed cells
Muscles
(DMSO)
Neurons
(RA)

Figure 13

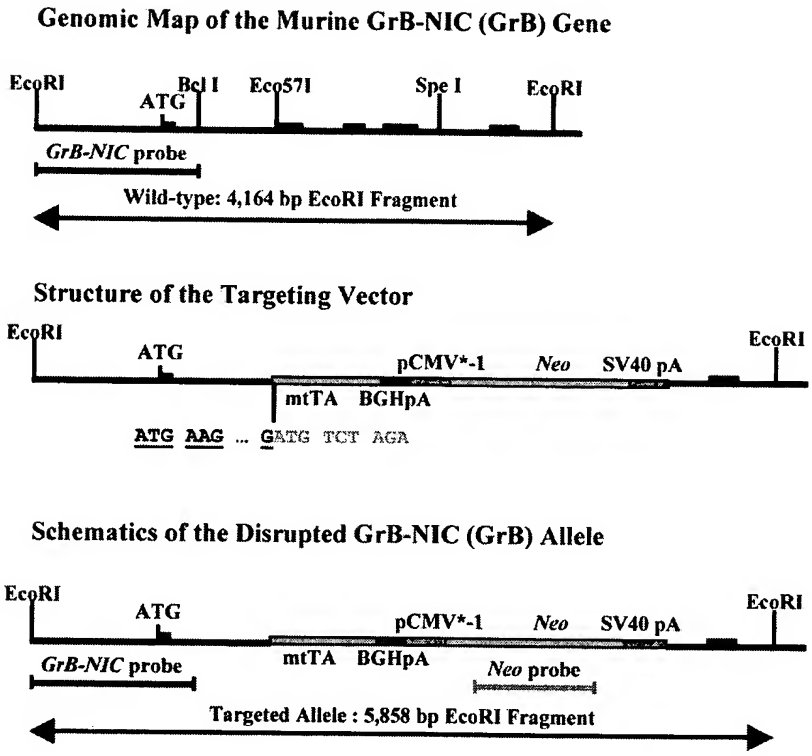
Genomic Map of the Murine GrB-NIC (GrB) Gene
EcoRI
ATG
BclI
Eco57I
SpeI
EcoRI
GrB-NIC probe
Wild-type: 4,164 bp EcoRI Fragment
Structure of the Targeting Vector
EcoRI
ATG
pCMV*-1
Neo
SV40 pA
EcoRI
mtTA
BGHpA
ATG AAG ... GATG TCT AGA
Schematics of the Disrupted GrB-NIC (GrB) Allele
EcoRI
ATG
pCMV*-1
Neo
SV40 pA
EcoRI
GrB-NIC probe
mtTA
BGHpA
Neo probe
Targeted Allele : 5,858 bp EcoRI Fragment

Figure 14

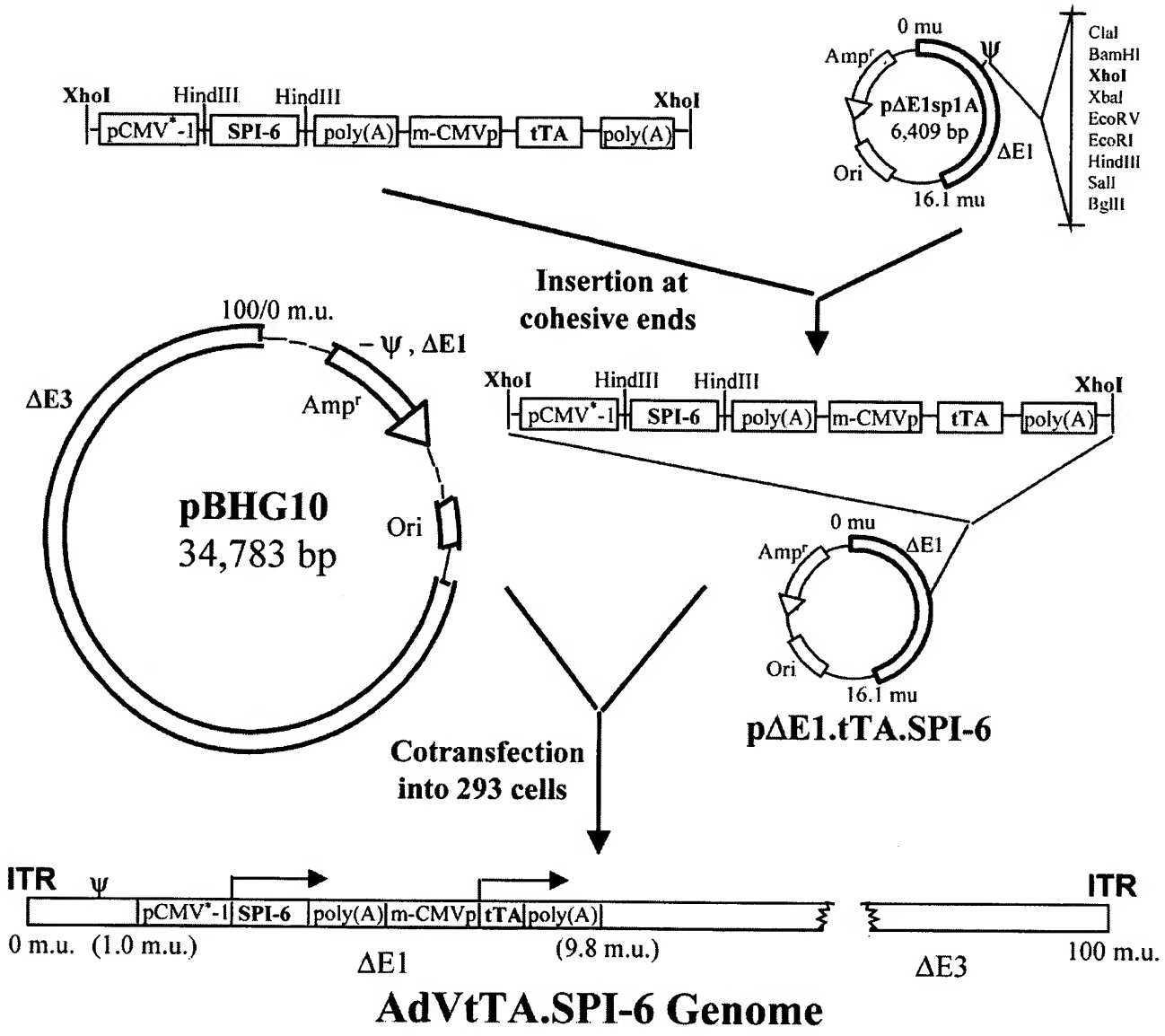
XhoI
HindIII
HindIII
XhoI
pCMV*-1
SPI-6
poly(A)
m-CMVp
tTA
poly(A)
0 mu
Ampr
pΔE1sp1A
6,409 bp
ΔE1
Ori
16.1 mu
ClaI
BamHI
XhoI
XbaI
EcoRV
EcoRI
HindIII
SalI
BglII
Insertion at
cohesive ends
100/0 m.u.
- ψ , ΔE1
ΔE3
Ampr
pBHG10
34,783 bp
Ori
XhoI
HindIII
HindIII
XhoI
pCMV*-1
SPI-6
poly(A)
m-CMVp
tTA
poly(A)
0 mu
ΔE1
Ampr
Ori
16.1 mu
pΔE1.tTA.SPI-6
Cotransfection
into 293 cells
ITR
ψ
pCMV*-1
SPI-6
poly(A)
m-CMVp
tTA
poly(A)
ITR
0 m.u.
(1.0 m.u.)
ΔE1
(9.8 m.u.)
ΔE3
100 m.u.
AdVtTA.SPI-6 Genome

Figure 15

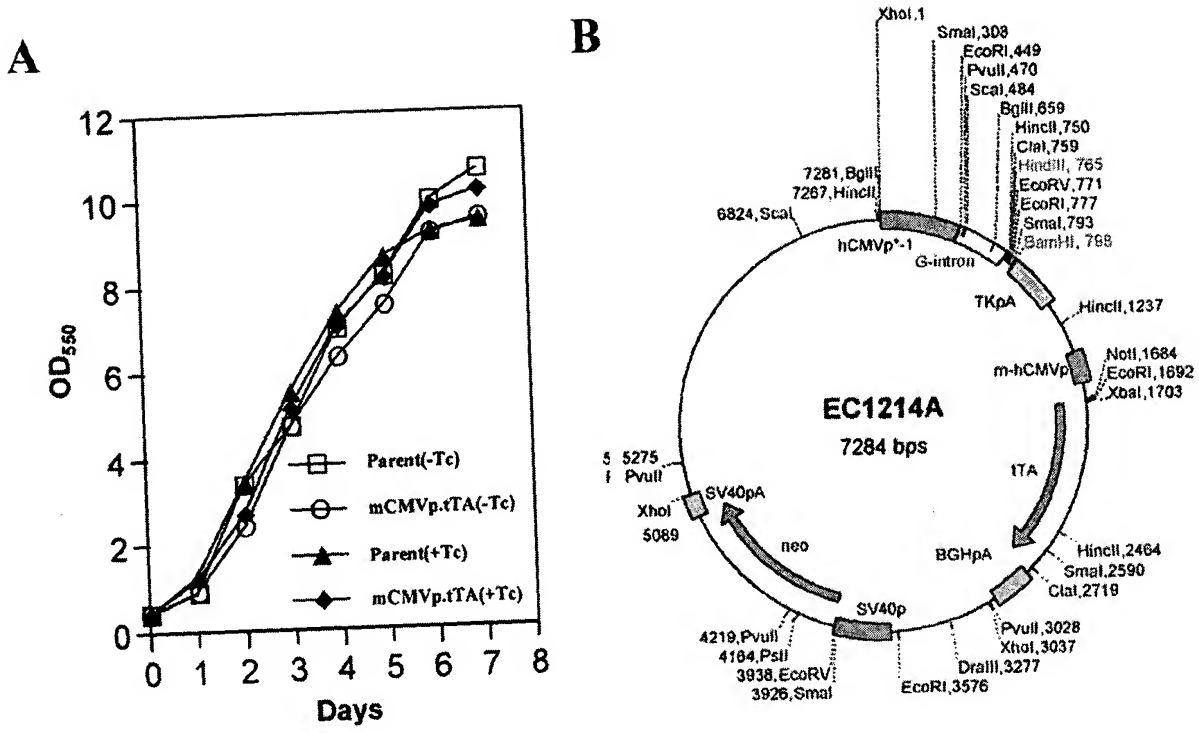
A
B
OD550
Days
Parent(-Tc)
mCMVp.tTA(-Tc)
Parent(+Tc)
mCMVp.tTA(+Tc)
EC1214A
7284 bps
XhoI,1
SmaI,308
EcoRI,449
PvuII,470
ScaI,484
BglII,659
HindIII,750
ClaI,759
EcoRV,771
EcoRI,777
SmaI,793
7281,BglII
7267,HindII
6824,ScaI
hCMVp*-1
G-intron
TKpA
HindIII,1237
NotI,1684
EcoRI,1692
XbaI,1703
m-hCMVp
tTA
BGHpA
HindIII,2464
SmaI,2590
ClaI,2719
PvuII,3028
XhoI,3037
DraIII,3277
EcoRI,3576
SV40p
4219,PvuII
4164,PstI
3938,EcoRV
3926,SmaI
neo
XhoI
5089
SV40pA
5275
PvuII

Figure 16

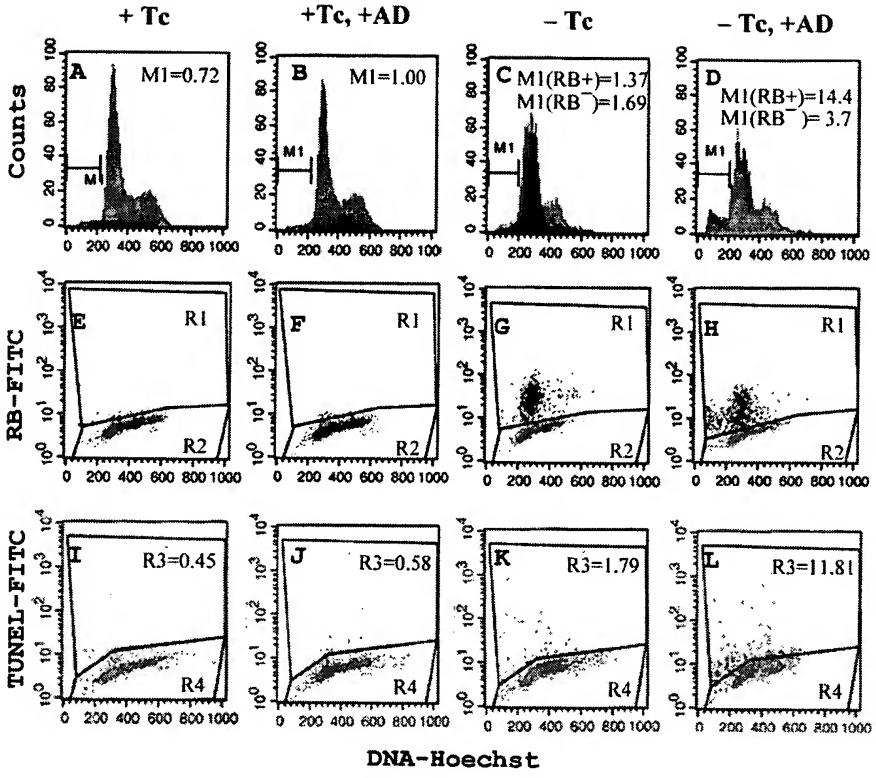
+ Tc
+Tc, +AD
– Tc
– Tc, +AD
Counts
A
M1=0.72
B
M1=1.00
C
M1(RB+)=1.37
M1(RB⁻)=1.69
D
M1(RB+)=14.4
M1(RB⁻)= 3.7
RB-FITC
E
R1
R2
F
R1
R2
G
R1
R2
H
R1
R2
TUNEL-FITC
I
R3=0.45
R4
J
R3=0.58
R4
K
R3=1.79
R4
L
R3=11.81
R4
DNA-Hoechst

Figure 17

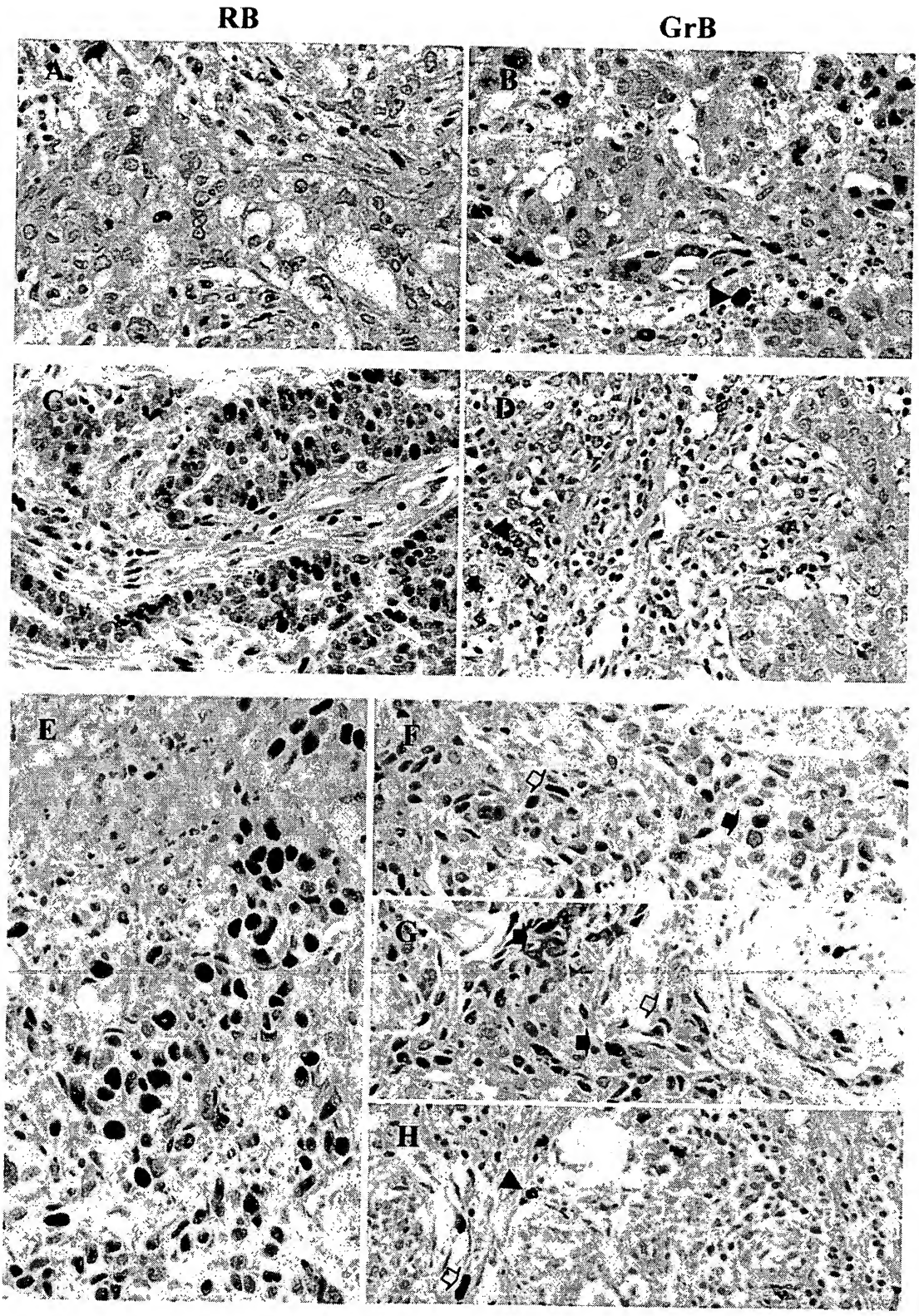

Figure 18

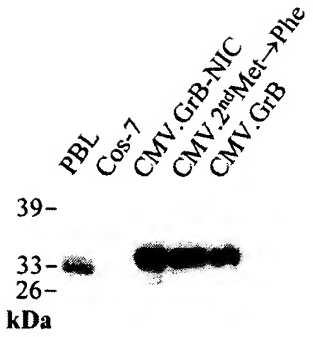

Figure 19

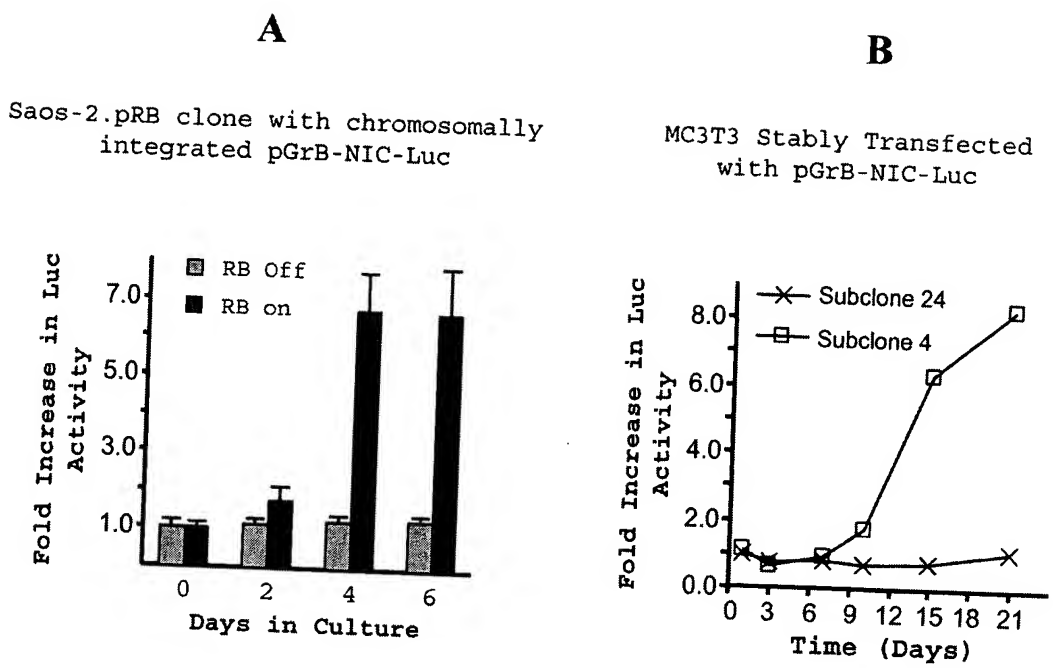
A
Saos-2.pRB clone with chromosomally integrated pGrB-NIC-Luc
RB Off
RB on
Fold Increase in Luc Activity
7.0
5.0
3.0
1.0
0
2
4
6
Days in Culture
B
MC3T3 Stably Transfected with pGrB-NIC-Luc
Subclone 24
Subclone 4
Fold Increase in Luc Activity
8.0
6.0
4.0
2.0
0.0
0 3 6 9 12 15 18 21
Time (Days)

Figure 20